ORIGINAL ARTICLE

Seroprevalence of and Risk Factors for *Toxoplasma gondii* in the US Swine Herd Using Sera Collected During the National Animal Health Monitoring Survey (Swine 2006)

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Impacts

- *Toxoplasma gondii* infects animals destined for human consumption, including pigs.
- ELISA serological mean within-herd prevalence of *T. gondii* in sera collected during the 2006 NAHMS swine survey was found to be 2.7% with a median within-herd prevalence of 0%.
- Using cats or dogs for rodent control, and specific carcass disposal methods were found to be risk factors for *T. gondii* infection in swine production sites.

Keywords:
Toxoplasma gondii; pigs; good production practices; food safety; seroprevalence

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Summary

The United States Department of Agriculture (USDA) initiated the National Animal Health Monitoring System (NAHMS) in 1983 to collect, analyse and disseminate data on animal health, management and productivity in US domestic livestock populations, including swine. The programme includes an on-farm serological sampling component which can be used to monitor seroprevalence of various pathogens, including *Toxoplasma gondii*. The purpose of this study was to determine the seroprevalence of *T. gondii* in grower/finisher pigs using sera collected during NAHMS Swine 2006 and to determine farm level factors associated with differences in seroprevalence on farms where sera was collected during the Swine 2006 survey. Sera and data on management practices for this study were collected from 185 grower/finisher swine production sites located in 16 states accounting for >90% of US swine production (Arkansas, Colorado, Iowa, Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Carolina, Ohio, Pennsylvania, South Dakota, Texas and Wisconsin). A total of 6238 sera were tested for *T. gondii* antibodies using a commercial ELISA assay (*Vet. Parasitol.* **128**, 2005, 177). Seroprevalence in this study, as determined by ELISA, was 2.6%, with a herd prevalence of 21.6% and a mean within-herd prevalence of 2.7%. Analysis of swine management practices indicated that rodent control methods and carcass disposal methods were associated with differences in the number of *T. gondii* positive samples on farm. These results are consistent with current epidemiological knowledge of the transmission of *Toxoplasma* on the farm (ingestion of organic matter containing oocysts, or ingestion of infected animal tissues). Production practices which eliminate these sources of exposure can reduce the risk of *Toxoplasma* infection in pigs, and reduce the likelihood of human infection from consumption of infected pork.

Introduction

Toxoplasma gondii is a coccidian parasite with an unusually wide range of intermediate hosts. Felids serve as definitive hosts and produce the environmentally resistant oocyst stage. *Toxoplasma* is one of the most common parasitic infections of man. In the United States, surveys have demonstrated that seroprevalence in people remained stable at 23% from 1990 until 1998 (Jones et al., 2001). Recent surveys have demonstrated a significant decrease in seroprevalence to 10.8% over the last decade (Jones et al., 2007). *Toxoplasma gondii* also infects food animals, including pigs. Infected animals harbour tissue cysts, and human consumers can be infected by ingestion of these cysts in raw or undercooked meat. Virtually all edible portions of an animal can harbour viable *T. gondii* tissue cysts (Dubey et al., 1986), and tissue cysts can survive in food animals for years. The exact contribution of food-borne toxoplasmosis to human infection in the US is unknown. A nationwide retail meat survey was recently completed which determined the prevalence of viable *T. gondii* tissue cysts in beef, pork and chicken (Dubey et al., 2005); only pork was found to harbour viable *T. gondii* tissue cysts, making pork a potential consumer concern. Several sensitive serological methods are available for testing swine for infection with *T. gondii*, including a commercial ELISA kit (Gamble et al., 2005; Hill et al., 2006). Results from the 1995 and 2000 NAHMS swine surveys reported seroprevalence in the US grower/finisher swine of 3.2% and 0.9%, respectively (Patton et al., 1998, 2002). In this study, we again measured the seroprevalence in grower/finisher pigs for *T. gondii*. We also analysed management practices associated with swine exposure to *T. gondii* using data collected during the 2006 NAHMS swine survey. These data are critical to building a foundation for the development of effective control programmes for *Toxoplasma* in domestic swine and for evidence-based education of swine producers, consumers, USDA regulatory agencies and international trading partners concerning the safety of pork products.

Materials and Methods

Initial questionnaire and biological sample selection

Serum samples were collected as part of the National Animal Health Monitoring System's (NAHMS) Swine 2006 study which focused on swine operations with 100 or more hogs. Information from the National Agricultural Statistics Service (NASS) Hog and Pig Report (dated 12/28/04) for numbers of hogs and pigs in each state, and the Farms and Land in Farms Report (dated 1/1/05) for number of operations with 100 or more hogs in each state was used to select the 17 states to be sampled for the study (http://www.nass.usda.gov). These 17 states accounted for 94.0% of the US swine inventory (as of 12/1/04) for operations with more than 100 hogs and 94.2% of the US operations with more than 100 hogs. Operations with 100 or more hogs from these 17 states that were on the NASS list frame were eligible to participate in the Swine 2006 study.

A stratified random sample of 5,000 operations was selected from the NASS list frame. Stratification was based on state and herd size, and larger operations were selected with a higher probability. All selected operations, with the exception of operations that were considered out of scope (University farms, research facilities and prisons), were eligible for an on-site interview with up to three questionnaires. Participation in the survey was voluntary, and participating farm identity was strictly confidential. All production sites surveyed maintained herds of at least 100 pigs as of the April 2006 NASS list frame estimates. Numbers of animals on farms surveyed by NASS enumerators on June 1, 2006, ranged from 0 to over 40 000, with an average of 2816 pigs per site. Pig management flow for grower/finisher pigs was typically all-in/all-out by building for the initial questionnaire. A total of 2230 producers completed the initial swine farm questionnaire, which included questions on number of animals in production phases, animal housing, biosecurity, animal and facility management and breeding practices. Two follow-up questionnaires (514 for the first and 435 for the second) were provided to participating producers, and 185 producers in 16 states (one state submitted no blood) with grower/finisher pigs agreed to allow blood samples to be taken from their herds. This voluntary response was low as compared with the original sample, thus the sample should be considered a convenient sample and the results presented here only represent this sample. Grower/finisher market pigs (approximately 20–32 weeks of age) were the target population for testing for *Toxoplasma gondii*. Four farms had blood sampled from 130 pigs that were less than 20 weeks of age, and four farms had blood sampled from 93 pigs that were greater than 32 weeks of age. The rest of the sampling (6015 samples) was completed within the target population. Within herd sampling was designed to give 95% confidence of detecting at least one positive animal, assuming a within-herd prevalence of at least 10%. As the ELISA test used to detect *Toxoplasma* is highly sensitive and specific (approximately 88.6% and 98%, respectively; Gamble et al., 2005), a maximum of 35 serum samples per farm was sufficient to meet the design requirements. This sample was also used to calculate a best estimate of within-herd prevalence given the number of samples collected from each site.

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Data and blood sample collection

Veterinary Medical Officers (VMOs) were provided with numbered kits containing 12.5 ml Vacutainer® separator tubes; 10 cm, 16 gauge needles; 20 ml syringes for collection of blood; tube labels and blood collection record sheets. At each pig production site, blood samples were taken from up to 35 pigs. Up to 13 ml of blood was collected from the cranial vena cava or jugular vein and injected into a Vacutainer® tube. Tubes were inverted 5 times to mix the clot activator, and allowed to sit vertically at 20–24°C for a minimum of 30 min. An 8-digit farm (state/operation/site), facility and pen ID was written on each tube label. Information recorded on the blood collection record sheet at the time of sample collection included farm ID, collection date, kit number, tube number, facility/building ID/pen ID, number of pigs in pen, number of pigs that share the same air space, pig age, pig gender and whether the sampled pig was vaccinated for PRRS, influenza, or *Mycoplasma*. Samples were cooled to 4°C immediately after clotting and shipped on ice within 24 h of collection to the USDA's National Veterinary Services Laboratories (NVSL) in Ames, IA. At NVSL, tubes were centrifuged and the sera drawn off, aliquoted and frozen. Aliquots for *Toxoplasma* antibody testing were sent to the USDA's Animal Parasitic Diseases Laboratory in Beltsville, MD, along with a copy of the Blood Collection Record. Serum samples were tested in duplicate for the presence of antibodies to *T. gondii* using a commercial ELISA, as recommended by the manufacturer (SafePath Laboratories, Carlsbad, CA, USA) (Hill et al., 2006). Sera were tested at a 1 : 50 dilution, and positive and negative control pig sera supplied by the manufacturer were included on each ELISA plate. ELISA values were reported as the mean optical density (OD) values of duplicate wells after subtraction of the OD value for the negative control well. Optical densities which exceeded 0.20 after subtraction of the negative control OD value were considered positive. All sera which tested positive in the initial ELISA were retested by ELISA to confirm the initial results.

Statistical analysis

Individual sample serological results were summarized in an Excel dataset and were merged with the Blood Collection Record dataset. This merged dataset was validated for missing information in SAS©, and collapsed to the farm level (6238 individual results to 185 herd-level results) producing a dependent variable of number of positive samples from each farm for use in inferential analysis. A dichotomous variable with either a 1 or a 0 value was also created, with 1 symbolizing one or more *Toxoplasma* positive samples occurring on a farm (farm positive *Toxoplasma* status). These would be used as dependent variables in inferential analysis. Based on a literature review, farm management factors potentially associated with *Toxoplasma gondii* infection were selected from each of the three questionnaires in the NAHMS Swine 2006 study. All continuous variables from the questionnaires to be used as independent variables in inferential analysis were changed to dichotomous variables as we were more interested in the impact of the presence of a variable. For example, the number of breeding animals was dichotomized because we were interested in determining whether having a breeding herd or not increased the number of *Toxoplasma* positives.

The final inferential analysis was carried out using PROC NLMIXED in SAS©. This procedure allows a zero-inflated model to be built. A zero-inflated model consists of two parts. The first part is often called the zero-inflated portion of the model and uses a binomial distribution to control for the probability of no event in the overall model. This would be the case for a farm with no *Toxoplasma* positives (zero counts). This first part may be performed using 3 different assumptions: the probability of excess zero counts is modelled as a constant (with a range of 0.1 to 0.9), the probability of excess zero counts is modelled as a function of independent variables, and the probability of excess zero counts is a constant function (Tau) of the mean predicted value of the count portion of the model's independent variables. The second part is often called the count portion of the model and uses a Poisson or Negative Binomial (or other) distribution to model the occurrence of counts of non-zero events as associated with independent variables. PROC NLMIXED zero-inflation modelling, therefore, usually involves a combination of binomial/Poisson (ZIP) or binomial/negative binomial (ZINB) distributions. After examining models using binomial/Poisson and binomial/negative binomial distributions and three different assumptions for the zero-inflated portion of the models for each, the final models selected were ZIP models. One assumed the probability of excess zero counts to be a constant and the other assumed the probability of excess zero counts to be a function of independent variables. These models were selected because of their predictive superiority (predicted farms that had various counts of positives versus actual number of farms that had various counts of positives). The outcome of analysis in the ZIP models was the count of positive samples for *Toxoplasma* antibodies divided by the number tested.

For the zero-inflated potion of the ZIP model that used an assumption that the probability of excess zero counts is a function of independent variables, PROC GENMOD and PROC LOGISTIC using a binomial distribution were

used to screen independent variables. For the count portion of the zero-inflated model, PROC GENMOD using a Poisson distribution was used to screen independent variables. In each case, all independent dichotomous variables were univariately screened for association with the dependent variable for a *P*-value of ≤0.2. Remaining independent variables were then modelled together using a backwards elimination process (*P*-value <0.05 to remain in the model).

In PROC GENMOD using a Poisson distribution, the Type 3 contrast option for covariates, Wald statistics for the Type 3 contrast of the covariates and the DSCALE option were used. The last option was used because the outcome was known to be over-dispersed so that the parameter covariance matrix and likelihood function could be adjusted by a scale parameter.

For the final ZIP model, fit was primarily assessed by predicted numbers of farms with each value of counts of *Toxoplasma* positive samples generated and compared with actual counts as the primary diagnostics due to limited accepted diagnostic capabilities in SAS©. Additionally, PROC PRINCOMP was run on covariates to assess collinearity. This model was additionally replicated in STATA© to compare estimates generated in SAS and to obtain the Vuong statistic which measures whether a zero-inflated component is justified.

Results

A total of 6238 samples from 185 farms were tested for antibodies to *Toxoplasma* by ELISA. Upon initial testing, antibodies were detected in 165 of the samples (2.6% individual animal prevalence-data not shown). There was a mean within-herd prevalence of 2.7% and a median within-herd prevalence of 0% (data not shown). Of the 130 samples taken from pigs less than 20 weeks old, three were seropositive (2.3%). Of the 93 samples taken from pigs greater than 32 weeks old, 14 were seropositive (15%). Fifty-four percent (89 of 165) of ELISA OD values in *Toxoplasma* positive samples were above 0.8 OD units. There were 40 sites with one or more positive samples resulting in a farm prevalence of 21.6%. Positive pigs were detected in 13 of the 16 states from which samples were collected (Arkansas, North Carolina and Texas had no positive samples).

For the zero-inflated portion of the ZIP model that used an assumption that the probability of excess zero counts is a function of independent variables, PROC GENMOD and PROC LOGISTIC using a Binomial distribution were used to screen independent variables. This resulted in an intermediary logistic regression analysis on the way to the final ZIP model, where the outcome was the presence or absence of any positive samples for *Toxoplasma* antibodies (e.g. the farm was positive for *Toxoplasma* or not). These logistic regression analysis results indicated that the odds of a farm being positive for *Toxoplasma* were 7.7 times higher when grower/finisher pigs were not housed in total confinement. The Pearson's chi-square statistic value divided by the models degree of freedom was close to 1, indicating a good fit; however, predictive ability was low (data not shown).

The outcome of number of *Toxoplasma*-positive counts per farm modelled using the ZIP method allowed investigation of associations between covariates and the prevalence of positive samples on *Toxoplasma*-positive farms while controlling for excess zero counts. There were two ZIP models that had the same count portion covariates. These two models used different assumptions in the zero-inflated component: the probability of excess zero counts was modelled as a constant (with a range of 0.1 to 0.9) and the probability of excess zero counts was modelled as a function of independent variables. Table 1 shows both models' zero-inflated portions (probability of excess zero counts as a constant and intercept probability of zero counts for the probability of excess zero counts modelled as a function of independent variables. All covariates in the latter were non-significant, except the intercept) and the count portion covariates common to both. These two final models had the best predictive power. These models revealed that when controlling for zero inflation and other count covariates, for a farm that buries dead weaned pigs off site (weandisp11-five farms did so), the count of *Toxoplasma* positives was 7.2 times higher than when they were not buried off site. When a farm composted dead preweaned pigs on site (preweandisp 10–26 farms did so), the occurrence of *Toxoplasma*

Table 1. Variables found significant at $P \leq 0.05$ using zero-inflated Poisson models constructed using a constant probability of zero counts or the probability of excess zero counts from variables significant from a previous regression using a binomial distribution (185 DF)

Parameter	Estimate*	SE	*P* value
Probability of excess zero counts	0.76	0.03	<0.0001
Intercept probability of zero counts	3.15	0.19	<0.0001
Intercept of the mean of the non-zero data	5.83	0.21	<0.0001
Preweandisp10	1.83	0.19	0.0021
Weandisp11	7.20	0.23	<0.0001
Manmethods	0.37	0.21	<0.0001

*All estimates except the probability of excess zero counts are raised to the base of 'e'. Vuong = 2.99
When preweandisp10 = 1, farm composts dead preweaned pigs on site.
When weandisp11 = 1, farm buries dead weaned pigs off site.
When manmethods = 1, farm uses traps, bait, poison, exterminator or some other method besides cats or dogs for rodent control.

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positives increased 83%, while the use of traps, bait, poison, exterminator or some other method besides cats or dogs for rodent control on the farm (manmethods-178 farms did so) reduced the prevalence of *Toxoplasma* positives 63%, holding all other fixed effects constant and controlling for zero inflation (Table 1). The Vuong statistic was greater than 1.96, indicating that modelling with a zero-inflated component was desirable.

Table 2 shows the predictive power of each of the models used in Table 1. The data show that 145 of 185 farms had no *Toxoplasma* positive samples. Despite overdispersion, the ZIP model predicted the number of farms with increasing counts best. The ZIP model nearly correctly predicted the number of farms where there were no positives, but was poorer at predicting the number of farms that had three to five seropositive pigs per farm. The number of positive samples on each farm was between 0 and 22. No model was adequate for predicting the few farms that had 17, 19 or 22 positives per farm.

Discussion

Serological surveillance of the national swine herd for *T. gondii* infection has been conducted at 5-year intervals since 1990 (http://nahms.aphis.usda.gov/). Only sows were sampled in 1990. Grower/finisher and sow/breeder populations were surveyed concurrently in 1995 and 2000. The 2006 swine biological sampling targeted grower/finisher swine, which is the source of most fresh pork consumed in the US. The initial 1990 survey documented a nearly 20% seroprevalence of *Toxoplasma* in the US sow population. A sampling of grower/finisher populations in Tennessee and North Carolina during that same period revealed a seroprevalence of 1–3% (Assadi-Rad et al., 1995; Patton et al., 1996). Risk factors associated with *Toxoplasma* infection identified during both of these surveys included swine raised outdoors, hogs raised on small farms and cats on the premises. Subsequent surveys have documented a decline in *Toxoplasma* seroprevalence in sows from 20% in 1990 to 15% in 1995 and to 6% in 2000 (Patton et al., 1996, 1998, 2002). In contrast, seroprevalence in grower/finisher swine has remained somewhat stable over that period. The seroprevalence in 1995 and 2000 was 3.2% and 0.9%, respectively (Patton et al., 2002), and 2.6% in 2006. Reduced seroprevalence in sow populations probably resulted from the large-scale movement of the swine industry towards total confinement rearing (~80%) and an emphasis on facility biosecurity, while the stable low seroprevalence of ~2.0% in grower/finisher pigs may reflect gaps in adherence to good production practices known to prevent exposure to *Toxoplasma* in confinement-reared pigs. H. R. Gamble, J. P. Dubey, and D. E. Hill (unpublished data) audited 58 pork production sites and documented persistent *Toxoplasma* infection in confinement-reared grower/finisher swine. Increased risk of infection was associated with the presence of domestic cats, feral cats and wildlife, as well as poor practices for disposal of swine carcasses. Increased compliance with production practices which reduced risk of exposure of pigs, including the introduction of barn-only boots in infected production sites, demonstrated a reduction in *Toxoplasma* prevalence on infected farms with all but 1 site becoming negative for *T. gondii* infection after three production cycles. These results demonstrate that good production practices, as identified using NAHMS data, can be implemented to greatly reduce the risk of exposure to *Toxoplasma* in confinement-raised pigs.

In this study, use of traps, bait, poison or an exterminator as a rodent control programme rather than using cats or dogs for rodent control was significantly associated with a reduced number of *Toxoplasma* seropositive samples on surveyed farms. Rodents serve as reservoirs of infection for *Toxoplasma* as well as other swine diseases (Dubey et al., 1995; Weigel et al., 1995), and therefore a multifaceted rodent control programme is essential for elimination of rodents and their associated pathogens in swine production facilities. Cats are not effective at

Table 2. Actual and predicted number of farms in the final models for each number of positive *Toxoplasma gondii* serological results per farm

Number of positives	Actual farm count	Predicted farm count
0	145	144.75
1	16	9.50
2	9	10.65
3	3	8.22
4	2	5.03
5	2	2.68
6	2	1.34
7	0	0.68
8	1	0.36
9	1	0.22
10	0	0.16
11	0	0.14
12	0	0.14
13	0	0.13
14	0	0.13
15	0	0.12
16	0	0.11
17	2	0.10
18	0	0.08
19	1	0.06
20	0	0.05
21	0	0.04
22	1	0.02
Total	185	184.72

rodent control in swine production facilities (Timm et al., 1987), and are the only source of infectious oocysts for environmental contamination which can lead to *Toxoplasma* infection in swine.

The practice of feeding dead weaned pigs to other animals, composting dead preweaned pigs on site or burying dead pigs outside of the home site as a means of carcass disposal also was associated with an increased number of *Toxoplasma* seropositive samples on surveyed swine production facilities. These management practices may impact the prevalence of *Toxoplasma* in surrounding sylvatic and peridomestic animal populations, leading to infections in peridomestic species (rodents, cats) whose home ranges may overlap with swine production facilities. Poor adherence to biosecurity practices may allow these peridomestic species access even to confined pigs, resulting in infection.

As documented in this survey, in NAHMS swine surveys since 1990, and in other recent studies (Assadi-Rad et al., 1995; Dubey et al., 1995; Weigel et al., 1995; Gamble et al., 1999; Lehmann et al., 2003), non-confinement housing is a significant risk factor that increases the probability of a farm having one or more samples positive for *Toxoplasma*. Increased transmission of *Toxoplasma* can occur in pigs reared in non-confinement management systems because these animals have increased exposure to infected wildlife, organic material and soil contaminated with cat faeces containing infectious oocysts. Gamble et al. (1999) reported swine seroprevalence of 47.4% in swine production facilities in the northeastern US, where pigs were managed largely in non-confinement systems. Viable *T. gondii* was isolated from 51 of 55 finisher pigs from a farm in Massachusetts (Dubey et al., 2002).

Previous studies have suggested that consumption of undercooked meat products containing *T. gondii* tissue cysts may account for a significant proportion of *Toxoplasma* infections in humans in the US (Mead et al., 1999; Roghmann et al., 1999). In a recent nationwide survey of retail chicken, beef and pork in the US, only pork was found to harbour viable *Toxoplasma* tissue cysts. Viable tissue cysts were isolated from 0.38% of pork samples, and 0.57% of samples had antibodies to *Toxoplasma* (Dubey et al., 2005). The northeast US had a higher number of positive pork samples than other regions of the country, reflecting the higher risk of pig infection due to regional management practices. The low prevalence of *Toxoplasma* infection in pork reported by the Dubey et al. (2005) study does not support the contention that pork contributes significantly to human infection in the US. However, nearly 100 million hogs are slaughtered for food in the US each year (USDA, National Agricultural Statistics Service, www.nas.usda.gov/Charts_and_Maps/Hogs_and_Pigs/pigs_e.asp). Based on the prevalence reported here, it is possible that up to 2.6 million *Toxoplasma* infected hogs could enter the US food chain each year. A single market weight hog (~113 kg) yields approximately 70 kg of meat, or 620 individual 113 g servings (http://www.pork.org/newsandinformation/quickfacts/statsSection08.pdf; pages 78–94); even if one half of the harvested meat is processed (salting, cooking, freezing, etc.) by methods which are known to kill *Toxoplasma*, nearly 820 000 000 individual 113 g servings of *Toxoplasma* infected pork could be available for consumption in the US each year. As the annual per capita consumption of pork in the US is 22.4 kg (49.4 lbs), ample opportunity exists for exposure to *Toxoplasma* from infected pork and pork products. An upsurge in consumer demand for 'organically raised', 'humanely raised' and 'free range' pork products has resulted in increasing numbers of hogs being raised in non-confinement systems (Honeyman et al., 2006). Swine producers have been recruited to produce animals for the organic market to fulfil a consumer demand that has increased 20% per year in sales since 1990 (Dimitri and Greene, 2002). National Organic Program (NOP) standards (http://www.ams.usda.gov/nop/) require that all organically raised animals must have access to the outdoors, including access to pasture for ruminants. Though 'humanely raised' and 'free range' products have standards that are less stringently defined, outdoor access is also considered a requirement for labelling. These practices substantially increase the risk of exposure of pigs to *Toxoplasma*.

Foodborne diseases are increasingly recognized in industrialized countries and consequently, are more of a concern to consumers. Large outbreaks of foodborne diseases are being reported and covered extensively in the media, and the severe impact on children, the aged and immunocompromised individuals has resulted in a heightened awareness of the consumer to the issue of contaminated food. Demands of consumers for pathogen-free meat products have focused the attention of government regulators and the meat industry on food safety, and the necessity to produce meat that is wholesome, safe and of high quality. Knowledge on the prevalence of *Toxoplasma* and associated herd-level risk factors gained from this analysis of the 2006 NAHMS swine survey can be used to develop industry initiatives (pre-harvest risk reduction programmes) to reduce the risk of exposure of consumers to infected pork.

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